An epidemiological study of blood pressure and metabolic phenotypes in relation to the Gβ_3 *C825T* polymorphism

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Background **The *825T* allele of the G-protein β_3-subunit gene is associated with increased intracellular signalling and adipogenesis in experimental studies. We studied the *C825T* polymorphism in relation to blood pressure, obesity and intermediate phenotypes in a Caucasian population.**

Methods **We genotyped 737 men and 775 women (participation rate, 64.3%) enrolled in a Belgian population study. Dichotomous phenotypes were tested for association with the *C825T* polymorphism by Fisher's exact test and multiple logistic regression. For continuous traits, we used analysis of covariance and generalized estimating equations.**

Results **The *T* allele (39.7 versus 29.1%) and *TT* genotype (16.1 versus 7.7%) were more prevalent in obese men than in non-obese men ($P \leqslant 0.01$). *TT* homozygous men, compared with *C allele* carriers, had higher daytime ambulatory blood pressure (mean systolic/diastolic differences, 3.6/2.5 mmHg; $P \leqslant 0.02$), higher body weight (2.7 kg, $P = 0.04$), greater risk of obesity (risk ratio, 1.90; $P = 0.005$), increased triceps skinfold thickness (2.3 mm, $P = 0.007$), higher serum insulin concentration (4.1 mU/l, $P = 0.006$), more insulin resistance ($P = 0.01$), and increased erythrocyte count (0.11×10^{12} cells/l, $P = 0.04$) and haematocrit (0.9%, $P = 0.02$). In women, haematocrit and erythrocyte count were also higher ($P \leqslant 0.03$) in *T* allele carriers, but other phenotypes were not correlated with the *C825T* polymorphism.**

Conclusion **Male and female carriers of the *T* allele at position 825 of the G-protein β_3-subunit gene have a slightly higher haematocrit and erythrocyte count. Male *TT* homozygotes have a higher blood pressure and are more obese and insulin-resistant than *C allele* carriers. We speculate that the higher blood pressure in *TT* homozygous men might arise via a metabolic pathway characterized by obesity and insulin resistance as well as via increased peripheral resistance secondary to the higher haematocrit. *J Hypertens* 21:729–737 © 2003 Lippincott Williams & Wilkins.**

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See editorial commentary page 683

Introduction

Guanine nucleotide regulatory proteins (G-proteins) are part of the intracellular signalling cascades [1]. Their activation is the principal mechanism through which stimulated heptahelical receptors generate changes in intracellular function [1]. Siffert and coworkers identified a common *C825T* polymorphism in exon 10 of the β_3-subunit of the heterotrimeric G-protein [2]. The *825T* mutant is associated with a splice variant, which shortens the protein by 41 amino acids and produces a gain of function [2]. Increased signalling by G-proteins stimulates adipogenesis [3] and may lead to obesity [4]. In addition, *C825T* is a candidate polymorphism for hypertension, because the mutation entails stimulation of the ubiquitously expressed Na^+/H^+ exchanger [5]. At the kidney level, this may increase Na^+ re-absorption and cause chronic volume expansion [6]. Prevention of intracellular acidosis may also lead to proliferation of vascular smooth muscle cells, vascular remodelling and increased peripheral arterial resistance [6]. However, published reports on the association between hypertension and the *825T* mutation produced inconsistent results [2,7–9].

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There is growing awareness that complex age-related disorders, such as hypertension, cannot be studied from an exclusive genocentric point of view, but only within their epidemiological context [10]. Statistical analyses should account for factors, such as gender, age, lifestyle, use of medications, and circadian variability. While allowing for these influences we studied, in the Flemish Study on Environment, Genes and Health Outcomes (FLEMENGHO) [11], the G-protein β_3-subunit *C825T* polymorphism in relation to blood pressure, obesity and intermediate phenotypes selected because they may be affected by the variant signalling protein.

Methods

Study population

The Ethics Committee of the University of Leuven approved the protocol of the FLEMENGHO study. From August 1985 until November 1990, a random sample of the households living in a geographically defined area of Northern Belgium [11] was investigated with the aim of recruiting an equal number of participants in each of six subgroups by sex and age (20–39 years, 40–59 years, and $\geqslant 60$ years). All household members with a minimum age of 20 years were invited to take part until the quota of their sex–age group had been fulfilled. To further study the role of genetic factors, from June 1996 until January 1998, nuclear families including children who were at least 10 years old, were recruited using the former participants (1985–1990) as index persons [11]. In 1998 and 1999, all previously enrolled participants were invited to donate a blood sample for DNA extraction.

The study population included 2064 persons [11]. The participation rate among the subjects contacted was 64.3%. The blood pressure of 24 participants was not measured. In 1998–1999, blood for DNA extraction could not be obtained from 403 former participants, because they did not consent ($n = 161$) or because they had died ($n = 180$), were terminally ill ($n = 28$), or had moved out of the area ($n = 34$). Because of delays between field and laboratory work or insufficient DNA amplification, 125 subjects were not genotyped. Thus, we included 1512 subjects in the present analysis.

Measurement of phenotypes

Study nurses followed the guidelines of the British Hypertension Society to measure the participants' sitting blood pressure five times consecutively at each of two home visits 4–6 weeks apart. For analysis, these 10 blood pressure readings were averaged. Hypertension was diagnosed if the mean was at least 140 mmHg systolic or 90 mmHg diastolic, or when patients were on antihypertensive medications. The average daytime blood pressure was determined from unedited ambulatory recordings with weights according to the time elapsed between successful readings. Validated oscillometric SpaceLabs 90202 or 90207 monitors (Redmond, Washington, USA) were programmed to obtain measurements at 20-min intervals for at least 12 h between 0800 and 2200 h. For both conventional and ambulatory blood pressure measurement, standard cuffs had a 12×24 cm inflatable portion, but if the upper arm girth exceeded 31 cm then cuffs with a 15×35 cm bladder were employed.

Weight and height were measured without shoes with the subjects wearing light indoor clothing. Subjects were classified as normal, overweight or obese if their body mass index was < 25.0, 25.0–29.9 or $\geqslant 30.0$ kg/m^2, respectively. Triceps skinfold was determined at the left arm using a Harpender caliper providing a constant pressure of 0.01 kg/mm^2 ($\pm$ 10%) at all openings of the 90 mm^2 anvils. We administered a validated [11] questionnaire to collect information about the subjects' medical history, use of medications, smoking habits, intake of alcohol, physical activity, and menstrual status of women. From the questionnaire data and body weight, we estimated energy expenditure due to physical activity.

In the interval between the two home visits, the participants collected a 24-h urine sample in a wide-neck plastic container for measurement of sodium, potassium and creatinine. A venous blood sample for the determination of plasma renin activity was obtained usually within 2 weeks of the urine collection. As measure of insulin resistance, the homeostasis model assessment index (HOMA) was calculated from blood glucose and serum insulin concentrations determined after an overnight fast [12]. Erythrocytosis was an erythrocyte count of $\geqslant 5.5 \times 10^{12}$ cells/l in men and $\geqslant 5.0 \times 10^{12}$ cells/l in women. The diagnosis of diabetes mellitus relied on the medical history confirmed by each subject's doctor, on blood glucose concentrations of $\geqslant 6.1$ mmol/l or $\geqslant 10.0$ mmol/l in fasting and non-fasting conditions, respectively, or on the observation of glucosuria (> 5.5 mmol/l) by dipstick test on a fresh urine sample.

Determination of the genotype

Genomic DNA was extracted from white blood cells. From the published DNA sequence of the G-protein β_3-subunit gene [13], we amplified a 268 base pair fragment incorporating the polymorphic site with use of two primers as described by Siffert and coworkers [2]. We have previously published the technical procedures, which we implemented for DNA amplification and subsequent measurement of the genotypes by allele-specific hybridization [8].

Statistical methods

For statistical analysis, we used SAS version 8.1 (SAS Institute, Cary, North Carolina, USA). Continuous

measurements with a skewed distribution were normalized by logarithmic transformation and represented by the geometric mean and its 95% confidence interval (95% CI). Proportions were compared by Fisher's exact test. In each gender separately, we first searched for possible confounders using stepwise multiple regression with the *P* value for co-variables to enter and stay in the model set at 0.15. We used multiple logistic regression to test the associations between dichotomous phenotypes and genotypes while controlling for co-variables. In logistic regression, genotypes were represented by dummy variables defined according to the deviation from the mean coding approach, which does not imply any genetic hypothesis [14]. Continuous traits adjusted for co-variables were first analysed by analysis of covariance. Because in men genetic heterogeneity among pathophysiologically related phenotypes consistently suggested a recessive pattern, we subsequently compared *TT* homozygotes only with *C* allele carriers, using the least-square mean option of the PROC GLM procedure. Finally, we repeated our analyses using generalized estimating equations [15,16] to allow for the non-independence of the observations among related subjects. In the PROC GENMOD procedure of the SAS package, we defined the intra-familial correlation matrices based on our own data and we adjusted for the same co-variables as in the analysis of co-variance.

Results

Characteristics of participants

The 1512 participants included 737 men and 775 women (Table 1). One hundred and forty-five (9.6%) participants were younger than 20 years, 489 were aged 20–39 years (32.3%), 618 (40.9%) were aged 40–59 years, and 260 (17.2%) were 60 years or older. Men and women had similar age distributions with medians (interquartile range) of 42.9 years (32.1–55.8 years) and 43.0 years (32.9–56.0 years), respectively ($P = 0.82$ for gender difference). The study sample consisted of 762 unrelated individuals including 35 spouse pairs without offspring, and of 203 families with one to seven children (327 parents, 423 offspring; median number of offspring per family, 2).

Table 1 Characteristics of participants

Characteristics	Men ($n = 737$)	Women ($n = 775$)
Clinical measurements		
Body height (cm)	173.7 ± 7.9	161.5 ± 6.9
Body weight (kg)	77.0 ± 13.0	65.6 ± 13.0
Systolic pressure (mmHg)*	125.5 ± 15.2	120.9 ± 16.9
Diastolic pressure (mmHg)*	77.0 ± 10.5	74.2 ± 10.3
Pulse rate (beats/min)	67.9 ± 9.6	71.1 ± 10.0
Physical activity (kcal/day)†	1.42 (1.34–1.51)	1.29 (1.22–1.37)
Daytime ambulatory measurements‡		
Systolic pressure (mmHg)	126.5 ± 10.5	122.1 ± 10.9
Diastolic pressure (mmHg)	77.0 ± 8.4	74.7 ± 7.6
Pulse rate (beats/min)	75.2 ± 11.0	80.7 ± 10.4
Urinary measurements§		
Volume (l/day)	1.49 ± 0.60	1.56 ± 0.68
Creatinine (mmol/day)	13.9 ± 3.7	9.4 ± 2.4
Sodium (mmol/day)	201 ± 70	162 ± 58
Potassium (mmol/day)	78 ± 30	64 ± 23
Number (%) on treatment		
Antihypertensive drugs	71 (9.6)	102 (13.2)
β-Blockers	42 (5.7)	58 (7.5)
Diuretics	25 (3.4)	57 (7.4)

Unless indicated otherwise, values are means ± standard deviation. *Individual values are means of 10 readings, five at each of two separate home visits. †Geometric mean with 95% CI for 644 men and 684 women. ‡Means of daytime (1000–2000 h) measurements in 716 men and 753 women. §720 men and 762 women collected a 24-h urine specimen.

Mean (standard deviation) body mass index was 25.5 kg/m^2 (3.9 kg/m^2) in men and 25.2 kg/m^2 (5.1 kg/m^2) in women ($P = 0.23$). Body mass index did not differ among smokers and non-smokers (25.3 versus 25.6 kg/m^2, $P = 0.26$), but was higher in subjects reporting alcohol consumption (26.2 versus 25.1 kg/m^2, $P = 0.0003$) or intake of β-blockers (27.6 versus 25.4 kg/m^2, $P = 0.0003$). Hypertension ($n = 352$, 23.3%), obesity ($n = 193$, 12.8%), diabetes mellitus ($n = 43$, 2.9%) and erythrocytosis ($n = 86$, 5.8%) were equally prevalent ($P > 0.28$) among men and women. Two hundred and thirty-two men (31.5%) were current smokers and 245 (33.2%) reported intake of alcohol. Among women, these numbers were 215 (27.7%) and 88 (11.4%), respectively. One hundred and forty-seven women (19.0%) used oral contraceptives and 14 (1.8%) took hormonal replacement therapy.

Genotype and allele frequencies

The *C825T* genotype frequencies were 47.3% ($n = 715$) for *CC*, 43.8% ($n = 662$) for *CT*, and 8.9% ($n = 135$) for *TT*, and did not deviate from Hardy–Weinberg equilibrium ($P = 0.30$). The *C* and *T* allele frequencies were 69.2 and 30.8%. According to Fisher's exact test, genotype and allele frequencies did not differ according to the presence or absence of hypertension (Table 2). These findings remained unaltered if 197 untreated white-coat hypertensive patients with normal daytime systolic and diastolic pressures (< 135/< 85 mmHg) were excluded from analysis. In men, but not in women, the frequencies of the *T* allele and the *TT* genotype were significantly higher in obese subjects than in the other participants ($P \leqslant 0.01$; Table 2).

Association analysis in men

Co-variables identified by stepwise regression analysis are presented in Table 3. Compared with the overall risk in men, *TT* homozygous men had a 1.90 (95% CI, 1.21–2.98; $P = 0.005$) higher risk of obesity (Fig. 1) and a 1.81 (95% CI, 1.03–3.17; $P = 0.04$) higher risk of erythrocytosis (Fig. 2). Conversely, men with the *CC* genotype had a lower risk of obesity [risk ratio, 0.61 (95% CI, 0.43–0.86); $P = 0.004$; Fig. 1].

In unadjusted analyses, the analysis of variance *P* values for the continuous phenotypes presented in

Table 2 Genotype and allele frequencies by sex and classes of blood pressure and body mass index

Characteristic	Number of subjects (%) with genotype				Number of alleles (%)		
	CC	*CT*	*TT*	$P^{\dagger}$	*C*	*T*	$P^{\dagger}$
Men ($n = 737$)							
Normotension*	256 (45.6)	261 (46.5)	44 (7.9)		773 (68.9)	349 (31.1)	
Hypertension*	91 (51.7)	68 (38.6)	17 (9.7)	0.17	250 (71.0)	102 (29.0)	0.47
Normal weight	162 (49.5)	140 (42.8)	25 (7.7)		464 (70.9)	190 (29.1)	
Overweight	153 (47.4)	148 (45.8)	22 (6.8)		454 (70.3)	192 (29.7)	
Obese	32 (36.8)	41 (47.1)	14 (16.1)	0.01	105 (60.3)	69 (39.7)	0.007
Women ($n = 775$)							
Normotension	286 (47.8)	250 (41.7)	63 (10.5)		822 (68.6)	376 (31.4)	
Hypertension	82 (46.6)	83 (47.2)	11 (6.2)	0.16	247 (70.2)	105 (29.8)	0.60
Normal weight	204 (47.8)	181 (42.4)	42 (9.8)		589 (69.0)	265 (31.0)	
Overweight	116 (47.9)	103 (42.6)	23 (9.5)		335 (69.2)	149 (30.8)	
Obese	48 (45.3)	49 (46.2)	9 (8.5)	0.80	145 (68.4)	67 (31.6)	0.87

*Classification based on the mean of 10 blood pressure measurements at home and World Health Organization/International Society of Hypertension limits. †Fisher's exact test was used to compare hypertensive with normotensive subjects as well as obese participants (body mass index $\geqslant 30.0$ kg/m^2) with those who had normal body weight (< 25.0 kg/m^2) or who were overweight (25.0–29.9 kg/m^2).

Table 3 Co-variables selected in stepwise regression

Phenotypes	Co-variables in men and women	Additional co-variables in women
Blood pressure at home and daytime ambulatory pressure	Age, age^2, smoking, alcohol intake, and antihypertensive treatment	Menopause, use of oral contraceptives
Body weight, body mass index and triceps skinfold thickness	Age, age^2, height (not considered for body mass index), smoking, alcohol intake, and use of β-blockers, diuretics or both	Use of oral contraceptives
Plasma renin activity	Age, body mass index, smoking, alcohol consumption, use of β-blockers and diuretics, time of day of blood sampling, urinary Na^+/K^+ ratio	Use of oral contraceptives
Erythrocyte count, haematocrit, plasma insulin, and insulin resistance	Age, body mass index, smoking, alcohol consumption, use of β-blockers and diuretics	Use of oral contraceptives

Smoking, alcohol intake, menopause and use of medications were coded 0 or 1 for condition absent or present.

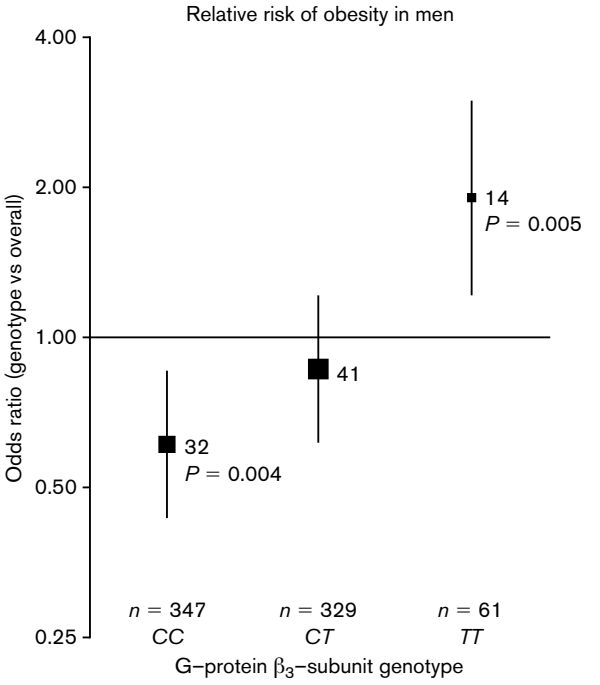
Table 4 ranged from 0.002 for plasma insulin concentration or insulin resistance up to 0.11 for systolic blood pressure at home. With full adjustments applied for the co-variables presented in Table 3, the analysis of variance P values in Table 4 were $\leqslant 0.06$ except for systolic pressure measured at home ($P = 0.12$). Because in men genetic heterogeneity among pathophysiologically related phenotypes consistently suggested a recessive effect of the *T* allele, we subsequently compared *TT* homozygotes with *C* allele carriers. *TT* homozygous men had elevated systolic (129.8 versus 126.2 mmHg, $P = 0.009$) and diastolic (79.3 versus 76.8 mmHg, $P = 0.02$) daytime ambulatory blood pressures, increased body weight (79.5 versus 76.8 kg, $P = 0.04$) and triceps skinfold thickness (14.7 versus 12.4 mm, $P = 0.007$), higher serum insulin levels (15.4 versus 11.3 mU/l, $P = 0.006$), more insulin resistance (HOMA index, 2.23 versus 1.66; $P = 0.01$), and increased erythrocyte count (4.99×10^{12} versus 4.88×10^{12} cells/l, $P = 0.04$) and haematocrit (45.8 versus 44.9%, $P = 0.02$). On the other hand, *TT* homozygotes compared with *C* allele carriers, had similar fasting blood glucose (5.1 versus 5.0 mmol/l, $P = 0.57$), plasma renin activity (0.49 versus 0.43 ng/l per s; $P = 0.21$), and urinary Na^+/K^+ ratio (2.90 versus 2.77, $P = 0.37$).

Using a multiple regression approach, we explored to what extent body mass index, haematocrit and *C825T* genotype explained the variance in daytime blood pressure. After adjustment for the co-variables presented in Table 3 as well as for reciprocal effects, body mass index, haematocrit and *C825T* genotype, respectively, explained 3.5% ($P < 0.0001$), 0.8% ($P = 0.02$) and 0.6% ($P = 0.04$) of systolic pressure (model $R^2 = 0.086$) and 1.5% ($P = 0.001$), 0.5% ($P = 0.06$) and 0.6% ($P = 0.04$) of diastolic pressure (model $R^2 = 0.198$).

Association analysis in women

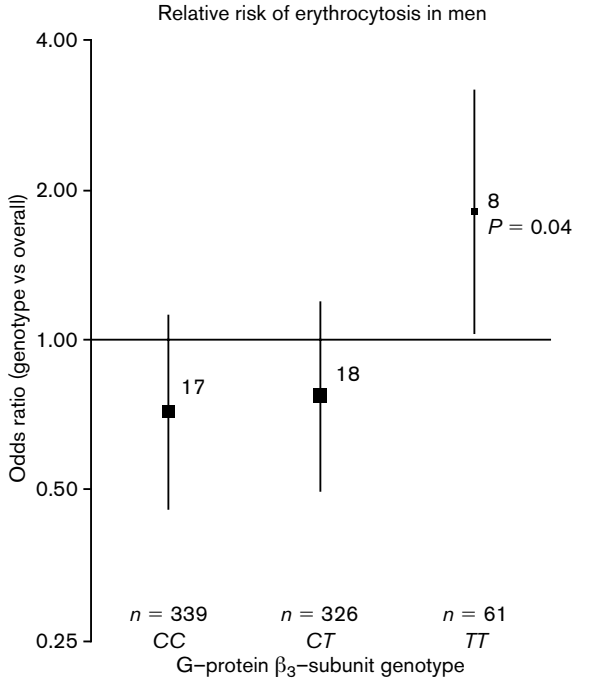
In women (Table 5), both before and after adjustment for the co-variables presented in Table 3, haematocrit ($P = 0.03$) and erythrocyte count ($P < 0.001$) were higher in *T* allele carriers, but none of the other

Fig. 1



Relative risk of obesity associated with the G-protein β_3-subunit genotype in men. The risk of obesity (body mass index $\geqslant$ 30 kg/m^2) is expressed relative to that in all men and is adjusted for age, age^2, smoking, alcohol intake, and the use of β-blockers and diuretics. Vertical lines denote 95% confidence intervals. For each genotype, the number of subjects is given. The size of the squares is proportional to the number of obese subjects given alongside the squares.

Fig. 2



Relative risk of erythrocytosis associated with the G-protein β_3-subunit genotype in men. The risk of erythrocytosis (red blood cell count $\geqslant 5.5 \times 10^{12}$ cells/l) is adjusted for age, body mass index, smoking, alcohol intake and the use of β-blockers and diuretics. Further explanation as in Fig. 1.

phenotypes was correlated with the *C825T* polymorphism. In particular, body mass index and daytime blood pressure were neither associated with the *T* allele in all women (Table 5) nor in subgroups defined on the basis of median age ($P > 0.27$), and/or multiparity ($P > 0.20$).

Sensitivity analyses

In men (Table 4) as well as in women (Table 5), the findings obtained by analysis of co-variance remained unaltered after exclusion of subjects on antihypertensive treatment or after additional forced adjustments for physical activity or the presence of diabetes mellitus. In addition, we also repeated our analyses with similar

Table 4 Adjusted phenotypes by G-protein β$_3$-subunit genotype in men

	Adjusted G-protein β_3-subunit genotypes						*P* values*	
	CC		*CT*		*TT*		Level of adjustment	
	n	Statistic	*n*	Statistic	*n*	Statistic	None	Full
SBP at home (mmHg)	347	125.6 ± 0.8	329	124.9 ± 0.8	61	128.2 ± 1.8	0.11	0.12
DBP at home (mmHg)	347	76.9 ± 0.5	329	76.5 ± 0.5	61	79.3 ± 1.2	0.08	0.05
Daytime SBP (mmHg)	336	126.7 ± 0.6	320	125.7 ± 0.6	60	129.8 ± 1.3	0.009	0.009
Daytime DBP (mmHg)	336	76.9 ± 0.4	320	76.6 ± 0.4	60	79.3 ± 1.0	0.04	0.02
Body weight (kg)	347	76.4 ± 0.5	329	77.3 ± 0.5	61	79.5 ± 1.3	0.06	0.04
Body mass index (kg/m^2)	347	25.3 ± 0.2	329	25.6 ± 0.2	61	26.3 ± 0.4	0.13	0.06
Triceps skinfold thickness (mm)	285	12.2 ± 0.4	266	12.6 ± 0.4	52	14.7 ± 0.8	0.008	0.007
Erythrocyte count (10^{12}/l)	339	4.90 ± 0.02	326	4.86 ± 0.02	61	4.99 ± 0.05	0.01	0.04
Haematocrit (%)	338	45.0 ± 0.2	326	44.7 ± 0.2	61	45.8 ± 0.4	0.009	0.02
Plasma insulin (mU/l)	266	11.8 (10.8–13.0)	257	10.7 (9.8–11.8)	51	15.4 (12.5–19.0)	0.002	0.006
Insulin resistance†	111	1.68 (1.53–1.84)	120	1.65 (1.51–1.81)	20	2.23 (1.78–2.79)	0.002	0.01

SBP, systolic blood pressure; DBP, diastolic blood pressure. Values are arithmetic means (standard error) or geometric means (95% confidence interval) adjusted for the co-variables presented in Table 3. *P values denote differences between *C* allele carriers and *TT* homozygotes. Full adjustment refers to the co-variables presented in Table 3. †Homeostasis model assessment index calculated only for fasting subjects as described in [12].

Table 5 Adjusted phenotypes by G-protein β_3-subunit genotype in women

	Adjusted G-protein β_3-subunit genotypes*					
	CC		*CT*		*TT*	
Characteristic	*n*	Statistic	*n*	Statistic	*n*	Statistic
SBP at home (mmHg)	368	120.3 ± 0.7	333	121.7 ± 0.8	74	120.3 ± 1.6
DBP at home (mmHg)	368	73.7 ± 0.5	333	74.6 ± 0.5	74	74.8 ± 1.1
Daytime SBP (mmHg)	363	121.7 ± 0.5	318	122.4 ± 0.6	72	122.5 ± 1.2
Daytime DBP (mmHg)	363	74.1 ± 0.4	318	75.4 ± 0.4	72	74.1 ± 0.9
Body weight (kg)	368	65.9 ± 0.6	333	65.2 ± 0.7	74	66.0 ± 1.4
Body mass index (kg/m^2)	368	25.3 ± 0.2	333	25.1 ± 0.3	74	25.4 ± 0.5
Triceps skinfold thickness (mm)	291	2.14 ± 0.04	262	2.18 ± 0.04	62	2.09 ± 0.09
Erythrocyte count (10^{12}/l)	357	4.40 ± 0.02	329	4.48 ± 0.02	74	4.51 ± 0.04
Haematocrit (%)	357	40.6 ± 0.2	329	41.2 ± 0.2	74	41.2 ± 0.4
Plasma insulin (mU/l)	276	11.9 (10.9–13.0)	250	12.1 (11.0–13.3)	58	11.8 (9.8–14.4)
Insulin resistance	124	1.64 (1.51–1.79)	111	1.63 (1.49–1.79)	26	1.64 (1.35–1.98)

SBP, systolic blood pressure; DBP, diastolic blood pressure. Values are arithmetic means (standard error) or geometric means (95% confidence interval) adjusted for the co-variables presented in Table 3. *Before and after adjustment for the co-variables presented in Table 3, *P* values for differences across genotypes were non-significant with the exception of erythrocyte count ($P < 0.001$) and haematocrit ($P = 0.03$).

sets of co-variables as mentioned earlier using generalized estimating equations instead of analysis of covariance to allow for the non-independence of the observations within families. Again, these analyses confirmed our results.

Discussion

Our epidemiological study tested '*a priori*' hypotheses concerning pre-defined phenotype–genotype associations. However, it was neither designed to disentangle complex pathophysiological mechanisms nor to prove causality. Nevertheless, our observations suggest a new working hypothesis concerning the association between hypertension and the *C825T* polymorphism. First, the mutated *825T* allele may increase blood pressure through a metabolic pathway characterized by the presence of obesity and insulin resistance [6]. Second, the higher haematocrit and erythrocyte count associated with the splice variant may raise blood pressure, because they increase blood viscosity and resistance to arteriolar blood flow [17] or because they increase oxygen delivery to peripheral tissues, thereby leading to arterial vasoconstriction [18].

Studies of possible association between various cardiovascular phenotypes and the *C825T* polymorphism of the G-protein β_3-subunit gene produced inconsistent results. The mutated *825T* allele was associated with higher blood pressure [7–9,19] or increased risk of hypertension [2,8,20,21] in Germans [2,7,9,20], Australian white hypertensive patients [19], and Caribbean or West African blacks [21], but not in French [8], Irish [8], Japanese [22,23], or African-Americans [24]. Few of the aforementioned studies reported analyses stratified by gender [9,19]. Only one smaller study in Canadian Oji-Cree noticed significantly lower systolic and diastolic blood pressures in *TT* homozygotes than in *C* allele carriers [25]. Among hypertensive patients, left ventricular hypertrophy [26] or impaired left ventricular diastolic filling [27] were linked with the mutated G-protein β_3-subunit allele in Spanish [26] and in Germans [27]. These discordant cardiovascular findings suggest that ethnic and genetic background [4], lifestyle and various environmental factors may modulate the small effect of the *C825T* allele on blood pressure. The frequency of the *T* allele was 0.31 in our Caucasian population, but approximately 0.50 in Japanese [22,23] or native Americans [25,28], and 0.75 in blacks [21,24]. Another reason for the divergent findings with regard to blood pressure may reside in the fact that this phenotype is more distant from the *C825T* genetic polymorphism than the intermediate haematological and metabolic factors, which may contribute to the rise in blood pressure, but for which many counter-regulatory mechanisms may compensate.

We observed thickening of the triceps skinfold, slightly higher body mass index and body weight and higher risk of obesity in *TT* homozygous men. Increased signalling by pertussis-toxin-sensitive G-proteins stimulates adipogenesis [3]. Published reports on the association between measures of obesity [4] and the G-protein β_3-subunit *C825T* polymorphism are more consistent than those on hypertension. Several studies [4] demonstrated that the *825T* homozygous genotype was positively associated with obesity or higher body mass index in blacks, Caucasians and Chinese. Primiparous *TT* homozygotes are at increased risk of obesity and post-pregnancy obesity [29]. Siffert speculated that the *825T* allele predisposes to obesity and hypertension via hyperinsulinaemia [6]. In line with this hypothesis, we found higher plasma insulin concentrations and more insulin resistance in *TT* homozygous men.

To the best of our knowledge, we are the first to report slight increases in haematocrit and erythrocyte count in

association with the G-protein β_3-subunit *825T* allele. Long-term increase in haematocrit leads to vasoconstriction-dependent hypertension, which is characterized by elevated cytoplasmic calcium concentration in vascular smooth muscle cells and by resistance to the vasodilatory action of nitric oxide [18]. Erythropoietin is the major regulator of the proliferation and differentiation of erythroid progenitor cells. Promotion of cell growth by erythropoietin [30,31] is primarily mediated via activation of the JAK/STAT pathway involving a specific set of cytosolic tyrosine kinases (JAKs) and transcription factors (STATs), and via stimulation of various protein kinase C isoforms. However, molecular studies also produced evidence for the involvement of heterotrimeric G-proteins in erythropoiesis [31–35]. G_i-proteins are associated with the C-terminal end of the erythropoietin receptor [32]. Activation of this receptor leads to dissociation of the G_i-protein, suggesting a functional role of the G_i-protein in signal transduction [32]. Upon stimulation of the erythropoietin receptor, G-proteins mediate a dose-dependent increase in cytosolic calcium via voltage-independent calcium channels [33]. Moreover, in human erythroid progenitor cells, G-proteins inhibit the antiproliferative effects of the activated thrombin receptor [31,34,35]. Thus, although the exact molecular pathways still need further clarification, experimental evidence supports the concept that the G-protein β_3-subunit *C825T* polymorphism may be associated with significant, albeit small, changes in erythropoiesis [31–35].

Adjustment for body mass index, haematocrit or both factors did not completely remove the effect of the *C825T* polymorphism on the daytime ambulatory blood pressure in men. Mechanisms other than those proposed might therefore also play a role. At the kidney level, stimulation of the Na^+/H^+ exchanger may increase Na^+ re-absorption and cause chronic volume expansion with a compensatory inhibition of plasma renin activity [6]. In keeping with this hypothesis [6], one population-based study [7] reported a significantly lower plasma renin activity in the presence of the *T* allele. Furthermore, Turner and colleagues [36] observed in patients with essential hypertension that systolic and diastolic blood pressures declined 5–6 mmHg more in response to diuretic treatment in *TT* homozygotes than in *CC* homozygotes. Zeltner and associates [5] found in young men with normotension or mild incipient essential hypertension that carriers of the *825T* allele had a faster tubular sodium re-absorption than *CC* homozygotes. Although we did not observe a significant decrease in plasma renin activity in *T* allele carriers, our study does not exclude chronic sodium retention [5,6] as a pathway underlying the higher blood pressure in *TT* homozygous men. Other possible mechanisms involve altered intracellular signalling after α-adrenergic or β-adrenergic receptor stimulation [1]. Current knowledge does not allow one to differentiate between the involvement of distinct G-protein subunits and their isoforms in pathophysiological processes, but the β_3-subunit is widely distributed across most tissues.

Before we engaged in the statistical analysis, we planned to analyse men and women separately. Indeed, blood pressure is an age-related trait with a gender-specific time course during life. Syndrome X is more prevalent among men than women [37]. Furthermore, several studies involving other known candidate genes revealed gender-specific associations with blood pressure. For instance, in two large-scale population studies [38,39], the risk of hypertension was related to the deletion polymorphism of the angiotensin-converting enzyme in men, but not in women. We previously found that, in our Flemish population, *CC* homozygosity at position 1797 of the β-adducin gene was associated with a lower risk of hypertension in women, but not in men [40].

Our sample size was sufficiently large to accommodate sex-specific analyses [16]. The biological consistency among men in the direction of the phenotypic changes associated with the *TT* genotype may be due to the intrinsic features of the metabolic syndrome [37]. On the other hand, the coherence and plausibility of the latter results can also be interpreted as evidence arguing against a type-1 error due to chance. Conversely, our null findings in women with regard to blood pressure and obesity might be due to a type II error. However, sample size calculations performed by Trégouët and colleagues [16] demonstrated that 650 unrelated subjects are sufficient to detect, with 90% power, an association with an allele, which has a frequency of 30% and explains 1.6% of the variance of a continuous phenotype. In similarly informed calculations involving either families with two offspring or mixed populations consisting of related and unrelated people, 650 subjects still provided 85–88% power, regardless of whether the analysis accounted for family structure. Sexual dimorphism exists in the heritability of blood pressure [41]. It is, therefore, conceivable that our null findings in women are due to a modification of the genetic effect, for instance by female sex hormones. Oestradiol increases inositol triphosphate formation via a signalling process that is linked to a pertussis-toxin-sensitive G-protein [42]. Moreover, oestradiol acutely and chronically behaves as a vasodilator [43].

Our findings did not evolve from a *post-hoc* hypothesis, neither in terms of the selected phenotype–genotype associations nor in terms of the separate analysis of men and women. Nevertheless, our results must be interpreted within the context of their limitations. They hinged on the differences between male *C* allele

carriers and *TT* homozygotes. Among men, the frequency of the *TT* genotype amounted to 8.3%. Only 20 *TT* homozygous men were available for the study of insulin sensitivity. Furthermore, with adjustments for co-variables applied, the increases in diastolic blood pressure, body weight, erythrocyte count and haematocrit in *TT* homozygous men were only borderline significant. *P* values are key in guiding statistical inference, but should also be interpreted within the context of pathophysiological plausibility [44]. With these limitations in mind, our findings should be viewed as hypothesis-generating and require further confirmation in sufficiently powered epidemiological studies, especially in populations with a higher prevalence of the *T* allele [4].

In conclusion, male and female carriers of the *T* allele at position 825 of the G-protein β_3-subunit gene have a slightly higher haematocrit and erythrocyte count. Male *TT* homozygotes have a higher blood pressure and are more obese and insulin-resistant than their *C* allele counterparts. We speculated that increased G-protein-mediated signalling upon stimulation of the erythropoietin or thrombin receptor of erythroid progenitor cells might explain the higher haematocrit and erythrocyte count associated with the *T* allele. Furthermore, the higher blood pressure in *TT* homozygous men might be brought about via a metabolic pathway characterized by obesity and insulin resistance as well as via increased peripheral arterial resistance secondary to the higher haematocrit.

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